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**A RAPID, PHYSIOLOGICALLY BASED METHOD FOR EVALUATING
CANDIDATE CHEMICAL WARFARE AGENT UPTAKE SIMULANTS**



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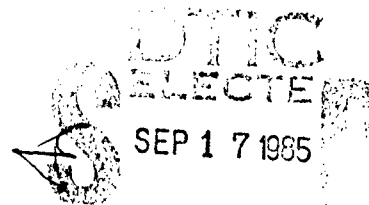
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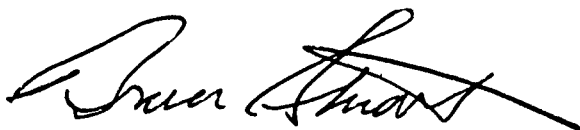
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FOR THE COMMANDER



BRUCE O. STUART, PhD
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| 19. ABSTRACT (Continue on reverse if necessary and identify by block number) Potential chemical warfare agent uptake simulants were selected and evaluated using a physiologically based method. Measurements of the partitioning of a chemical in body tissues and its physical properties, together with animal exposures in a closed chamber system interpreted by kinetic modeling, provided a rapid means of assessing the conformance of a particular chemical to criteria established for a useful simulant. A potential chemical warfare agent uptake simulant should as a minimum (1) have physical properties that allow for generation of realistic exposure conditions for the agent being simulated, (2) have good detectability, (3) be very poorly metabolized (biologically inert), and (4) be safe to humans in the concentrations to be encountered. Perfluorochemicals representing both aliphatic and aromatic compounds were used as initial candidates since they afford excellent detectability and relative biological inertness. Analogs of the initial compounds were subsequently tried until satisfaction of the established criteria was obtained. The required changes in chemical structure were driven by predictions from a | | | | | |
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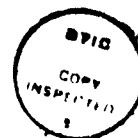
physiologically based computer model. The main thrust of this report is to outline the application of physiological modeling to the important task of chemical warfare agent uptake simulant selection (design). Chloropentafluorobenzene was identified as a potential uptake simulant for non-persistent chemical warfare agents.

PREFACE

This research was performed in the Biochemical Toxicology Branch, Toxic Hazards Division, Harry G. Armstrong Aerospace Medical Research Laboratory, from June 1984 through February 1985. The research was performed in support of Project 2729, "Aerospace Biotechnology for Chemical Warfare Defense;" Task 272908, "Chemical Defense Toxicology;" Work Unit 27290801, "Mechanisms of Chemical Agent Toxicity."

The authors thank Colonel Michael G. MacNaughton for valuable assistance in the design of this project, Dr. Christ Tambourski for providing fluorocarbon chemicals used in the experiments, and SRA Gregory Sudberry and Mr. Scott Griffith for technical assistance in chemical property and physiological partition coefficient determinations.

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INTRODUCTION

Effective military operations in chemical warfare environments are largely dependent on the extent and quality of chemical warfare training received by military personnel. Objective evaluation of individual protective equipment, collective protection facilities, and individual proficiency in use of military equipment can be enhanced by use of appropriate chemical agent uptake simulants. As a minimum, a chemical warfare agent uptake simulant should fulfill the following criteria:

1. Have physical properties that allow generation of realistic exposure atmospheres of the chemical agent to be simulated.
2. Have good detectability characteristics for biological and environmental monitoring of the chemical.
3. Be as biologically inert as possible so that expired air or body fluid chemical concentrations can be quantitatively related to a chemical exposure.
4. Be safe to humans in the concentrations to be used.

The U. S. Air Force contracted with Arthur D. Little, Incorporated, to prepare a candidate chemical agent uptake simulant list (AFAMRL-TR-82-87) based on chemicals that have had widespread human use without associated adverse health effects, e.g., chemicals from the FDA's Generally Recognized as Safe (GRAS) List (Arthur D. Little, Inc., 1982). The most attractive candidates were dipentene, methyl benzoate, n,n-diethyl-m-toluanide, and dimethyl methyl phosphonate. All of these candidates failed to satisfy the minimal criteria cited above for an acceptable uptake simulant. The primary deficits occurred in the areas of good detectability and biological inertness. Rather than continue the approach of trying to identify a chemical from the GRAS list as a potential uptake simulant, an alternative approach was adopted: the requirements for a chemical agent uptake simulant would be well defined and then a chemical would be chosen based on meeting the established criteria. In particular, the selection of a chemical as a candidate simulant would be based on properties such as partitioning of the chemical between tissues, air and blood, resistance to extensive metabolism in the body, and the physical characteristics essential to the delivery of realistic concentrations to the testing environment. The assessment of metabolic characteristics was accomplished using laboratory animal testing and physiological modeling. Physiological modeling provides a mathematical description of the absorption, distribution, metabolism, and excretion of a chemical with respect to a particular biological system.

Since good detectability and relative biological inertness are two of the most important criteria for a chemical agent uptake simulant, perfluorochemicals were the first compounds investigated. These chemicals provide excellent detectability (0.01 ppm) with gas chromatography and electron capture detection. Additionally, perfluorocarbons have been extensively studied in an effort to design and optimize a synthetic blood substitute (Degani, 1982, and Clark, 1978). These studies have shown that perfluorocarbons are not extensively metabolized since increased fluoride concentrations are not found in the urine following administration (Yokoyama et al., 1978). The vapor pressures of the

perfluorocarbons do vary considerably with the structure of the chemical; however, generation of a perfluorocarbon training atmosphere should not be difficult.

The goal of this work was to describe a selection protocol which identifies candidate chemical warfare agent uptake simulants based on established physical, chemical, and biological criteria. This study did not address the logistical or economic feasibility of these potential candidates or the toxicity testing protocols for chemicals identified for further consideration in this selection process.

METHODS AND MATERIALS

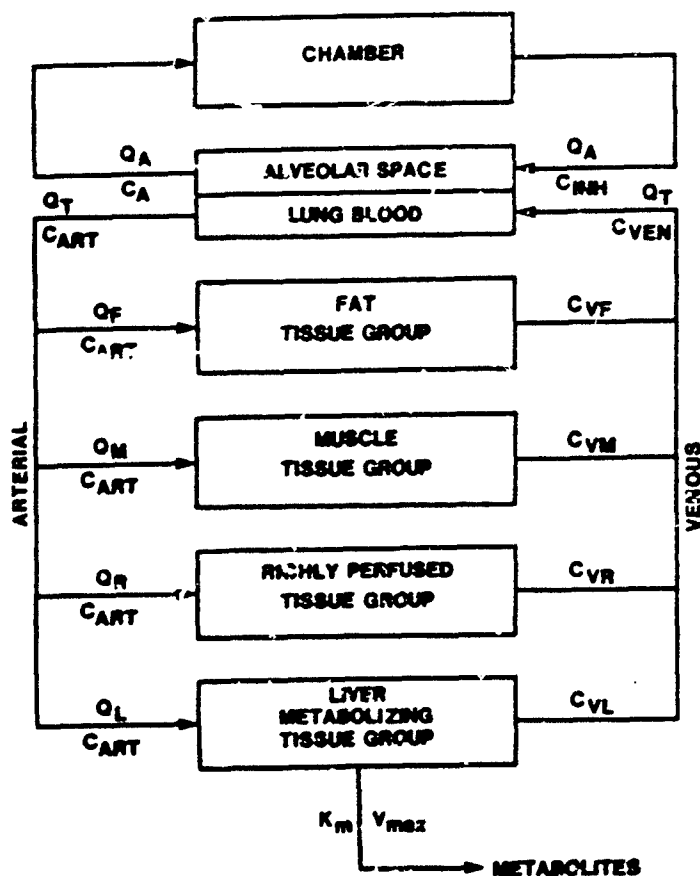
Chemicals. Perfluoropentane, perfluorohexane, perfluoroheptane, perfluorodecalin, and perfluorobenzene were purchased from PCR Research Chemicals, Incorporated, Gainesville, Florida. The pentafluorobenzene, bromopentafluorobenzene (BPFB), and chloropentafluorobenzene (CPFBB) were supplied by Dr. Christ Tambourski, Air Force Wright Aeronautical Laboratories, Wright-Patterson AFB, Ohio. The pyrazole (98% purity) was purchased from Aldrich Chemical Company.

Partition Coefficients. The blood:air, fat:air, liver:air, and muscle:air partition coefficients were determined using a modification of a vial equilibration technique (Sato and Nakajima, 1979). The technique involves headspace sampling after the chemical reaches equilibrium between the headspace and the homogenate (or blood). The headspace samples were analyzed using a Hewlett-Packard 5880 gas chromatograph with a flame ionization detector and a 1/8" diameter, 8' long stainless steel column packed with 10% SE-30 on Chromasorb W. One (1) mL headspace samples were injected into the column for analysis.

Closed Chamber Studies. The uptake (closed chamber) studies were conducted following the procedure described by Gargas and Andersen, 1985. Three to four animals were placed into a 9.1 L closed chamber and the volume of chemical required to produce the desired initial concentration was added to the chamber. The chemical concentration in the chamber was monitored every 10 minutes with an automatic sampling valve and a flame ionization detector on a Hewlett-Packard 5790 gas chromatograph. The HP 5790 used a 1/8" diameter, 10' long stainless steel column packed with 10% SE-30 on Chromosorb W. The oven temperature was 125°C. The chamber concentrations of 1 and 10 ppm were achieved by injecting a gas sample prepared in a sample bag into the chamber. The sampling of the chamber concentration at these low levels was done by manual injections (0.1 mL) into a Hewlett-Packard 5890 gas chromatograph equipped with a ⁶³Ni electron capture detector. The column used for the analysis was prepared as in the analysis using the flame ionization detector. A plot of chamber chemical concentration against time was prepared. The disappearance of chemical over time reflects partitioning of the chemical into the body tissues, the metabolism of the chemical, and the loss of the chemical by adsorption to the exposure chamber. The first phase of the curve describes partitioning and the second phase is descriptive of the metabolism (Andersen et al., 1978).

Computer Simulation/Physiological Modeling. The chemical concentration data generated in the uptake chamber were interpreted by means of a computer

simulation of the chemical exposure. The description of the animal system used in the physiological model is shown below.



In this illustration, Q_A is the alveolar ventilation rate, C_A is the alveolar concentration, C_{INH} is the chamber concentration, and C_{ART} and C_{VEN} are the concentrations in the arterial and venous blood, respectively. Q_R , Q_F , Q_L , and Q_M are the blood flows to the respective tissues, C_{VF} , C_{VM} , C_{VR} , and C_{VL} are the chemical concentrations in the venous blood leaving the respective tissues, and Q_T is total cardiac output. Differential equations (mass balance equations) are used to account for the amounts of chemical entering and leaving a particular tissue or organ compartment. The general form of the equations describing the change in chemical concentration with respect to time is illustrated in the following equation which corresponds to the liver compartment:

$$\frac{d(Amt_L)}{dt} = Q_L C_{ART} - Q_L C_L / R_L - \frac{V_{max} (C_L / R_L)}{K_m + C_L / R_L} - k_{fc} \cdot V_L \cdot C_L / R_L$$

where $Q_L C_{ART}$ is the amount of chemical entering the liver in arterial blood, $Q_L C_L / R_L = Q_L C_{VL}$ is the amount of chemical leaving in the venous blood, and

$$\frac{V_{\max} (C_L/R_L)}{(K_m + C_L/R_L)} - k_{fc} \cdot V_L \cdot (C_L/R_L)$$

is the amount of chemical metabolized within the liver. V_{\max} is the maximum velocity of the saturable metabolic pathway and K_m is the free chemical concentration where the velocity of the reaction is one-half the maximum velocity. The first order component of metabolism is described by the term $k_{fc} \times V_L (C_L/R_L)$ where k_{fc} is the first order rate constant (time^{-1}) and V_L is the volume of liver. Similar equations without terms for metabolism are written to describe the other compartments in the computer model. The temporal change in amount of chemical in the chamber is given by the equation:

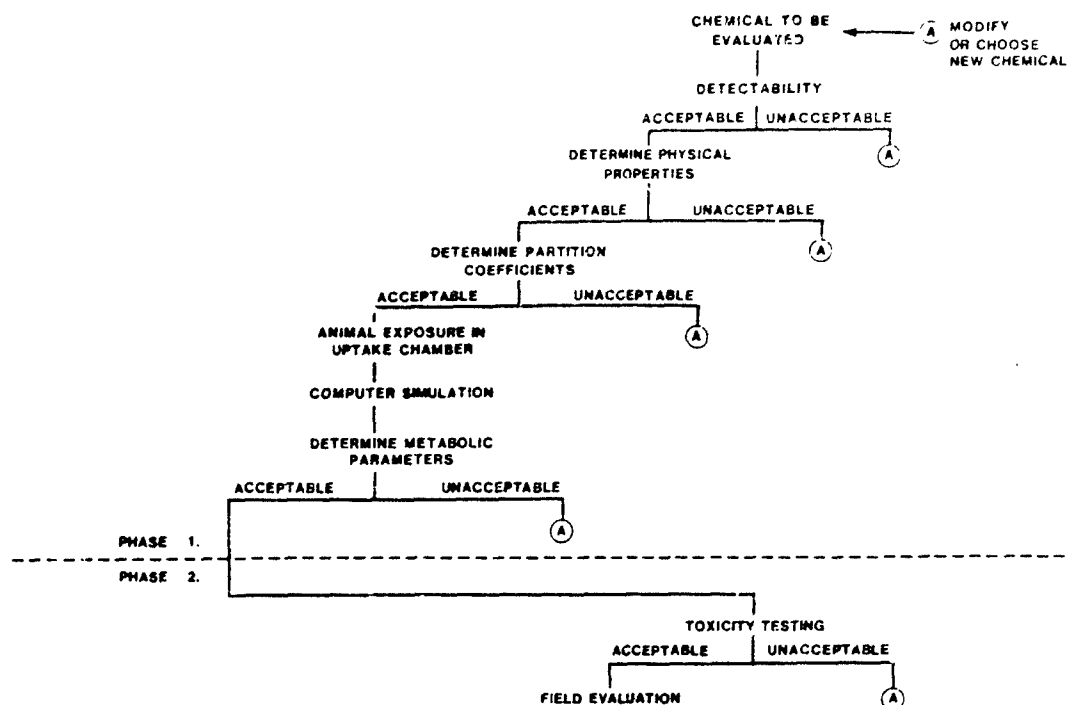
$$\frac{d(\text{Amt})}{dt} = R \times Q_p (CA/PB - CI) - (KL \cdot AI)$$

In this equation R is the number of rats, Q_p is the pulmonary blood flow (L/hr), CA is the alveolar concentration, PB is the blood:air partition coefficient for the chemical, CI is the concentration in inhaled air, KL is the rate of loss to the chamber system (hr^{-1}), and AI is the amount of chemical in the chamber. The metabolic constants in the liver portion of the model can be varied to produce a fit to the experimental data. The set of metabolic constants (V_{\max} , K_m , and k_{fc}) that fits experimental data for several runs over a range of exposure concentrations can be used to assess the metabolism of the chemical being evaluated.

Physical Constants. Boiling point and density measurements were made for all of the chemicals evaluated. The volatilities (mg/m^3) for selected chemicals were determined by analyzing (with GLC) an atmosphere saturated with the chemical at 25°C . The saturated atmosphere was produced by placing 0.1 mL of liquid into a 5.0 mL glass scintillation vial which was sealed with a septum screw cap. The volume of liquid added was sufficient to ensure that liquid remained in the vial at all times. The vial was placed into a heating block which was maintained at 25°C . After 48 hours a headspace sample was analyzed by gas chromatography for the chemical concentration in the vapor. The concentration in the headspace was converted to standard volatility units (mg/m^3). The vapor pressure was calculated from the volatility via the ideal gas law.

Selection Scheme. The overall scheme used for the evaluation of the chemicals for adherence to the criteria is shown at the top of the following page. The performance of a chemical at each branch point in the scheme determined whether further testing would be performed.

Evaluation Scheme for Candidate Chemical Uptake Simulants



RESULTS

Physical Properties. The boiling points, densities, vapor pressures, and volatilities are displayed in Table 1. Bromopentafluorobenzene and chloropentafluorobenzene had vapor pressures at 25°C of 3.76 mmHg and 14.1 mmHg, respectively.

Table 1. PHYSICAL PROPERTIES OF HALOCARBON
CANDIDATE SIMULANTS

| COMPOUND | CHEMICAL FORMULA | MOLECULAR WEIGHT | BOILING POINT(°C) | DENSITY (g/ml) | VAPOR PRESSURE (mm Hg, 25°C) |
|-------------------------------|----------------------------------|---------------------|----------------------|-------------------|------------------------------------|
| PERFLUOROPENTANE | C ₅ F ₁₂ | 288 | 30-32 | 1.61 | 646 |
| PERFLUOROHXANE | C ₆ F ₁₄ | 336 | 57 | 1.66 | 263.0 |
| PERFLUOROHEPTANE | C ₇ F ₁₆ | 384 | 80-82 | 1.69 | 92.5 |
| PERFLUORODECALIN | C ₁₀ F ₁₈ | 462 | 143 | 1.96 | 5.77 |
| PERFLUOROBENZENE | C ₆ F ₆ | 186 | 78 | 1.59 | 85.6 |
| BROMO- PENTAFLUOROBENZENE | C ₆ HBrF ₅ | 247 | 135 | 1.94 | 3.76 |
| CHLORO- PENTAFLUOROBENZENE | C ₆ ClF ₅ | 202 | 117 | 1.66 | 14.1 |

Partition Coefficients. The partition coefficients for the chemicals evaluated are presented in Table 2. Straight chain perfluorocarbons and perfluorodecalin had blood:air partition coefficients less than 1.0 as measured using the vial equilibration technique. Because these chemicals have very low blood solubilities and are rapidly exhaled, they would not be retained in the body for a sufficient time to allow post-exposure sampling. The aromatic fluorocarbons had higher blood:air partitions as well as considerably higher fat:air partition coefficients than the aliphatic compounds. The chemical with the highest partition coefficient in all of the tissues tested was bromopentafluorobenzene.

**Table 2. HALOCARBON TISSUE: AIR
PARTITION COEFFICIENTS ***

| <u>CHEMICAL</u> | <u>BLOOD:AIR</u> | <u>FAT:AIR</u> | <u>LIVER:AIR</u> | <u>MUSCLE:AIR</u> |
|-------------------------------|------------------|----------------|------------------|-------------------|
| PERFLUOROPENTANE | 0.22 ± 0.1 | 15.7 ± 1.0 | 26.8 ± 2.8 | 27.2 ± 1.0 |
| PERFLUOROHXANE | < 0.1 | 5.4 ± 0.3 | 14.5 ± 1.3 | 5.25 ± 1.0 |
| PERFLUOROHEPTANE | < 0.1 | 6.1 ± 0.6 | 10.9 ± 0.8 | 5.4 ± 0.5 |
| PENTAFLUOROBENZENE | 4.1 ± 0.1 | 245.0 ± 14.0 | 12.1 ± 1.9 | 9.0 ± 0.9 |
| PERFLUOROBENZENE | 2.6 ± 0.1 | 182.0 ± 3.0 | 14.6 ± 1.0 | 4.3 ± 0.6 |
| PERFLUORODECALIN | < 0.1 | 30.8 ± 0.7 | 10.6 ± 1.1 | 1.3 ± 0.1 |
| BROMO- PENTAFLUOROBENZENE | 32.9 ± 0.4 | 857.0 ± 44.0 | 71.5 ± 4.0 | 53.5 ± 3.0 |
| CHLORO- PENTAFLUOROBENZENE | 13.5 ± 0.5 | 766.0 ± 16.4 | 30.6 ± 0.5 | 34.6 ± 1.0 |

* ALL VALUES ARE MEAN ± COEFFICIENT OF VARIATION

Closed Chamber Uptake Studies. Chamber concentrations of bromopentafluorobenzene vs. time were obtained for initial concentrations of 2137, 855, and 214 ppm and all were essentially biphasic (Fig. 1). In the first phase (0-2 hours), the chamber concentration dropped rapidly. In the 2137 ppm run 91% of the initial concentration had been removed from the chamber, and in the 855 and 214 ppm runs, 89% and 95% of the initial concentrations had been removed by the animals, respectively. The second phase (2-6 hours) demonstrated a much slower, relatively constant rate of disappearance of chemical from the chamber atmosphere. Figure 2 shows the plots of chamber concentration for naive and pyrazole treated (320 mg/kg) rats. As can be seen, the pyrazole had no apparent effect on the concentration profile when compared to the naive rats. Pyrazole is an inhibitor of oxidative metabolism and can be used to determine the proportion of metabolism resulting from oxidative pathways inhibited by pyrazole (Anderson et al., 1978).

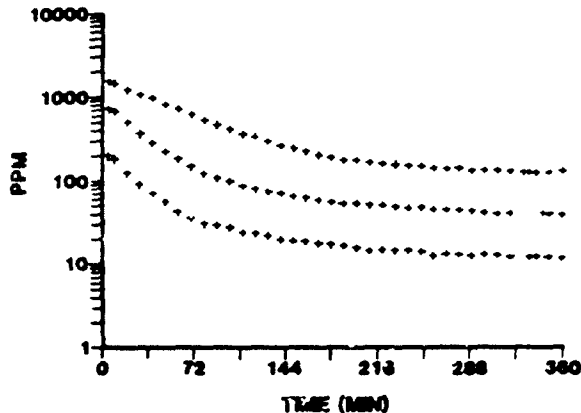


Figure 1. CONCENTRATION PROFILES FOR BROMOPENTAFLUOROBENZENE EXPOSURES

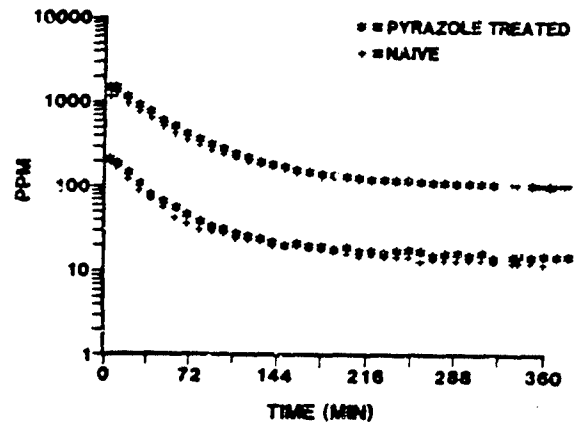
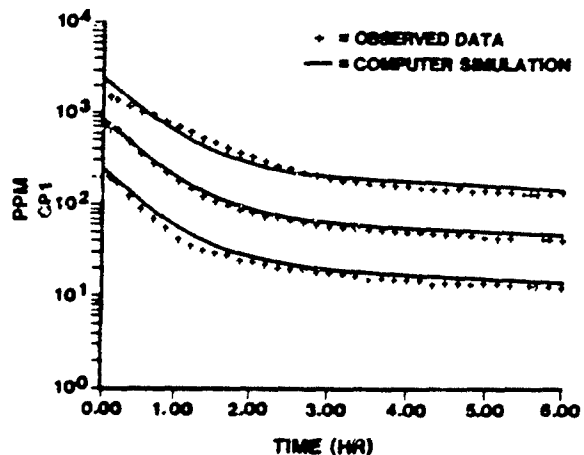


Figure 2. PYRAZOLE PRETREATED VS NAIVE RATS IN BPF8 EXPOSURE

The best fit computer simulation of the bromopentafluorobenzene exposure used a first-order metabolic constant of 6.0 hr^{-1} and no saturable component. The loss rate to the chamber was $0.0773/\text{hr}$ for the first hour and decreased to near zero after 4 hours. The computer simulation prediction of concentration profiles compared to the observed concentration profiles is shown in Figure 3. The loss of chemical is proportional to the chemical concentration in the chamber and is consistent with a first order pathway for metabolism.

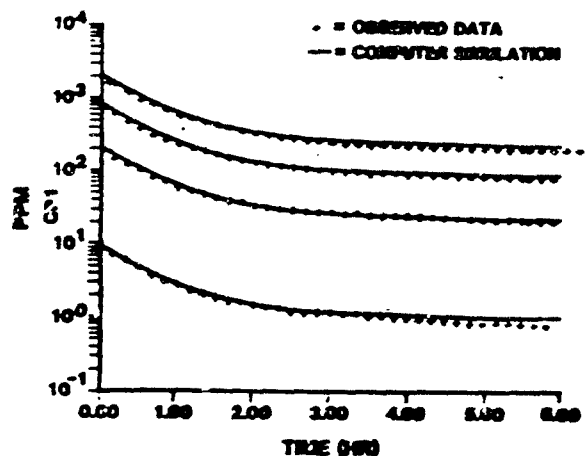
Figure 3. COMPUTER SIMULATION VS OBSERVED DATA FOR RATS EXPOSED TO BROMOPENTAFLUOROBENZENE IN A CLOSED CHAMBER SYSTEM



The computer simulation of the chloropentafluorobenzene exposure is shown in Figure 4 along with the observed data. The equilibrium phases of the chloropentafluorobenzene curves were more accurately described by the simulation than were those of the bromopentafluorobenzene curves. The concentration profiles for chloropentafluorobenzene were also biphasic with the first phase ranging from 0-2 hours and second phase ranging from 2-6 hours. However, the loss of initial concentration in the first phase was 84%, 82%, 84%, and 84% for 2137, 855, 214, and 10 ppm, respectively. The chloropentafluorobenzene also appeared to be metabolized by a first order mechanism; however, the rate constant was only about

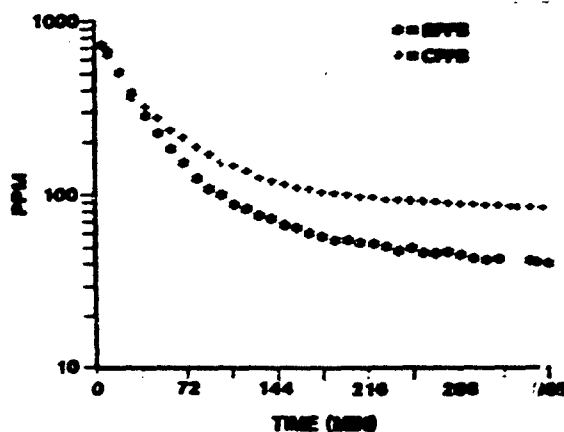
1/3 that of bromopentafluorobenzene. The computer simulation agreed with observed data when the first-order metabolic constant was 2.0 hr^{-1} with no saturable component.

Figure 4. COMPUTER SIMULATION VS OBSERVED DATA FOR RATS EXPOSED TO CHLOROPENTAFLUOROBENZENE IN A CLOSED CHAMBER SYSTEM



A comparison of the bromopentafluorobenzene and chloropentafluorobenzene exposures is illustrated in Figure 5. The lower partition coefficients for chloropentafluorobenzene in all of the measured tissues is consistent with the reduced uptake of chloropentafluorobenzene relative to the bromopentafluorobenzene during the equilibration phase (Phase 1). Similarly, Phase 2 of Figure 5 illustrates the relative biological inertness of chloropentafluorobenzene when compared to bromopentafluorobenzene. The slope of the concentration profile ($\Delta \text{concentration} / \Delta \text{time}$) is lower for the chloropentafluorobenzene than the bromopentafluorobenzene during the second phase (the phase describing metabolism).

Figure 5. COMPARISON OF BPFB AND CPFB CONCENTRATION PROFILES

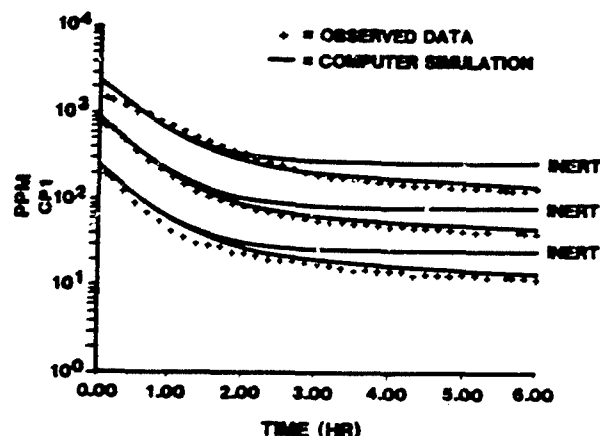


DISCUSSION

The identification of chloropentafluorobenzene as a potential chemical warfare agent uptake simulant resulted from the methodical application of physiological modeling and biological experimentation to evaluate compliance with

predetermined criteria. In this particular application, several aliphatic perfluorocarbons were chosen as initial candidates because of their sensitivity to detection by gas chromatography with electron capture detection and the reported biological inertness of the perfluorocarbon chemicals. The physical properties were determined and the vapor pressures of all of the tested chemicals were high enough to readily permit the generation of useful vapor concentrations. The next step in the evaluation was to determine the partition coefficients in the various biological tissues. The blood:air partition coefficients of less than 1.0 for the aliphatic fluorocarbons led to their elimination since the low blood:air partition would preclude detection of the chemical in blood at long times. To retain the exceptional detectability afforded by the fluorocarbons and increase the blood solubility, an aromatic perfluorocarbon was evaluated (perfluorobenzene). Perfluorobenzene had a blood:air partition coefficient of approximately 2. Although an improvement, the blood solubility was still too low to allow for acceptable post-exposure biological monitoring since the perfluorobenzene would be rapidly expired. To increase the blood solubility to acceptable levels a polar analog was obtained. Instead of a perfluorinated benzene, one of the fluorines on the aromatic ring was replaced by bromine (bromopentafluorobenzene). This chemical had a blood:air partition coefficient of 32.9 and also had increased detectability since the bromine responds more strongly than fluorine to electron capture detection. At this point a chemical with acceptable physical properties, good detection, and acceptable blood solubility has been identified. The next step in the process was to determine the extent of metabolism of the chemical. Animals were exposed in the closed uptake chamber and the observed data were compared to computer simulations describing the exposure. The physiological model and computer simulation allowed assignment of the biochemical constants descriptive of the rates of metabolism of the chemical. Unlike perfluorocarbon chemicals which are purportedly unmetabolized, bromopentafluorobenzene was metabolized to a limited extent as is shown in Figure 6 (Yokoyama et al., 1975).

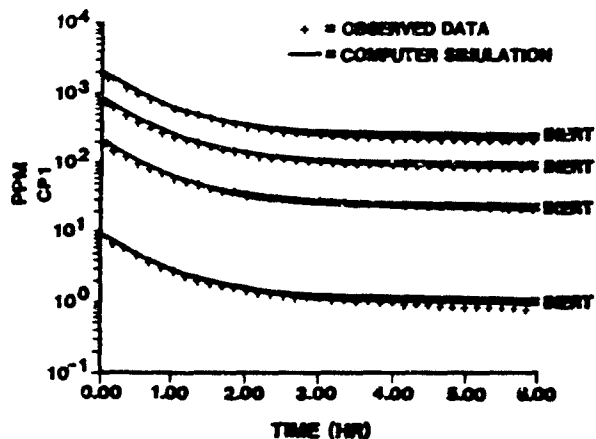
Figure 6. COMPUTER SIMULATION OF BIOLOGICALLY INERT COMPOUND VS OBSERVED DATA FOR RATS EXPOSED TO BROMOPENTAFLUOROBENZENE IN A CLOSED CHAMBER SYSTEM



Since bromopentafluorobenzene improved the adherence to the established criteria over perfluorobenzene except for the biological inertness criterion, a compound with the bromine replaced with a chlorine was evaluated. The rationale for using the chlorine analog was that since the carbon-fluorine bond on the aromatic ring was reportedly biologically inert and the carbon-bromine bond was vulnerable to attack in vivo, perhaps a carbon-chlorine bond (chlorine is a poorer leaving group

than bromine) would impart the desired solubility and detection characteristics of bromopentafluorobenzene and the biological inertness of perfluorobenzene. As discussed earlier, the chloropentafluorobenzene provided good detectability, desirable partitioning in biological tissues, acceptable physical properties, and relative biological inertness (Fig. 7).

Figure 7. COMPUTER SIMULATION OF BIOLOGICALLY INERT COMPOUND VS OBSERVED DATA FOR RATS EXPOSED TO CHLOROPENTAFLUOROBENZENE IN A CLOSED CHAMBER SYSTEM



The method described in this report provides a quick, reliable means of evaluating or screening chemicals as potential chemical warfare agent uptake simulants. The design of an uptake simulant using physical properties, computer simulations, and closed chamber exposures to ascertain adherence to established criteria allows for rapid identification of a chemical with optimized physical and biochemical characteristics. Additionally, modifications in structure can be quickly evaluated for compliance to established requirements or compared against other potential candidates for selection of the best possible uptake simulant.

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